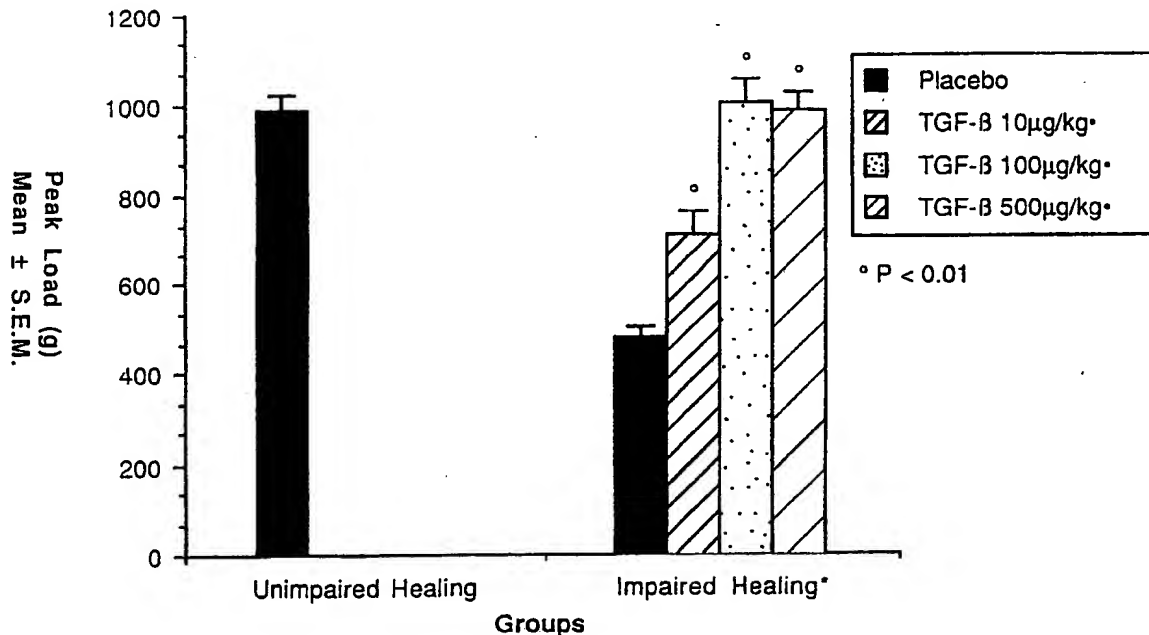




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(54) Title: METHOD OF PREDISPOSING MAMMALS TO ACCELERATED TISSUE REPAIR



## (57) Abstract

A method of predisposing a mammal to accelerated tissue repair is provided. This method comprises systemically administering to the mammal, prior to its exposure to tissue damage, an effective amount of TGF-β. Preferably, the TGF-β is administered no more than about 24 hours prior to the tissue damage.

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Description

## METHOD OF PREDISPOSING MAMMALS TO ACCELERATED TISSUE REPAIR

Technical Field

5 This invention relates to a method of predisposing mammals, especially humans, to accelerated tissue repair. More particularly, this invention is directed to a method of treating a mammal with transforming growth factor-beta before tissue injury to accelerate repair of the tissue.

Background Art

10 The beta transforming growth factors (TGF- $\beta$ s) are multifunctional cytokines, produced by many types of cells, including hematopoietic, neural, heart, fibroblast, and tumor cells, that can regulate the growth and differentiation of cells from a variety of tissue origins (Sporn et al., Science, 233: 532 (1986)) and stimulate the formation and elaboration of various stromal elements.

There are at least five forms of TGF- $\beta$  currently identified, TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, 15 TGF- $\beta$ 4, and TGF- $\beta$ 5. Suitable methods are known for purifying this family of TGF- $\beta$ s from various species such as human, mouse, green monkey, pig, bovine, chick, and frog, and from various body sources such as bone, platelets, or placenta, for producing it in recombinant cell culture, and for determining its activity. See, for example, R. Derynck et al., Nature, 316:701-705 (1985); European Pat. Pub. Nos. 200,341 published December 10, 1986, 20 169,016 published January 22, 1986, 268,561 published May 25, 1988, and 267,463 published May 18, 1988; U.S. Pat. No. 4,774,322; Cheifetz et al, Cell, 48: 409-415 (1987); Jakowlew et al., Molecular Endocrin., 2: 747-755 (1988); Dijke et al., Proc. Natl. Acad. Sci. (U.S.A.), 85: 4715-4719 (1988); Derynck et al., J. Biol. Chem., 261: 4377-4379 (1986); Sharples et al., DNA, 6: 239-244 (1987); Derynck et al., Nucl. Acids. Res., 15: 3188-3189 25 (1987); Derynck et al., Nucl. Acids. Res., 15: 3187 (1987); Derynck et al., EMBO J., 7: 3737-3743 (1988); Seyedin et al., J. Biol. Chem., 261: 5693-5695 (1986); Madisen et al., DNA, 7: 1-8 (1988); and Hanks et al., Proc. Natl. Acad. Sci. (U.S.A.), 85: 79-82 (1988).

The activated form of TGF- $\beta$ 1 is a homodimer formed by dimerization of the carboxy-terminal 112 amino acids of a 390 amino acid precursor (Derynck et al., Nature, *supra*). 30 Recombinant TGF- $\beta$ 1 has been cloned (Derynck et al., Nature, *supra*) and expressed in Chinese hamster ovary cells (Gentry et al., Mol. Cell. Biol., 7: 3418-3427 (1987)).

TGF- $\beta$ 2 has a precursor form of 414 amino acids and is also processed to a homodimer from the carboxy-terminal 112 amino acids that shares approximately 70% homology with the active form of TGF- $\beta$ 1 (Marquardt et al., J. Biol. Chem., 262: 12127 (1987)). TGF- $\beta$ 2 35 has been purified from porcine platelets (Seyedin et al., J. Biol. Chem., 262: 1946-1949 (1987)) and human glioblastoma cells (Wrann et al., EMBO J., 6: 1633 (1987)), and recombinant human TGF- $\beta$ 2 has been cloned (deMartin et al., EMBO J., 6: 3673 (1987)).

TGF- $\beta$ 3, TGF- $\beta$ 4, and TGF- $\beta$ 5, which are the most recently discovered forms of TGF- $\beta$ , were identified by screening cDNA libraries. The putative protein products of these three genes have not been isolated from natural sources, although Northern blots demonstrate expression of the corresponding mRNAs. Human and porcine TGF- $\beta$ 3 have been cloned and described previously (Derynck et al., EMBO J., **7**: 3737-3743 (1988), ten Dijke et al., Proc. Natl. Acad. Sci. USA, **85**: 4715 (1988)). TGF- $\beta$ 4 and TGF- $\beta$ 5 were cloned from a chicken chondrocyte cDNA library (Jakowlew et al., Molec. Endocrinol., **2**: 1186-1195 (1988)) and from a frog oocyte cDNA library, respectively. The frog oocyte cDNA library can be screened using a probe derived from one or more sequences of another type of TGF- $\beta$ . TGF- $\beta$ 4 mRNA is detectable in chick embryo chondrocytes, but is far less abundant than TGF- $\beta$ 3 mRNA in developing embryos or in chick embryo fibroblasts. TGF- $\beta$ 5 mRNA is expressed in frog embryos beyond the neurula state and in Xenopus tadpole (XTC) cells.

TGF- $\beta$  has been shown to have numerous regulatory actions on a wide variety of both normal and neoplastic cells. TGF- $\beta$  is multifunctional, as it can either stimulate or inhibit cell proliferation, differentiation, and other critical processes in cell function (Sporn et al., *supra*). For a general review of TGF- $\beta$  and its actions, see Sporn et al., J. Cell Biol., **105**: 1039-1045 (1987), Sporn and Roberts, Nature, **332**: 217-219 (1988), and Roberts et al., Recent Progress in Hormone Research, **44**: 157-197 (1988).

Natural TGF- $\beta$ 1 is made predominantly, if not exclusively, in a biologically latent form, which can be activated *in vitro* by denaturants such as urea, heat, plasmin, high salt, endoglycosidase F, capthepsin D, type IV collagenase, cocultured endothelial cells and pericytes, plasminogen activators such as urokinase, stimulated osteoclasts, or extremes of pH. See, e.g., Pircher et al., Canc. Res., **44**: 5538-5543 (1984) re latent TGF- $\beta$  from nontransformed and Kirsten sarcoma virus-transformed normal rat kidney cells; Antonelli-Orlidge et al., Proc. Natl. Acad. Sci. USA, **86**: 4544-4548 (1989) re latent TGF- $\beta$  from pericytes and capillary endothelial cells; Lawrence et al., Biochem. Biophys. Res. Commun., **133**: 1026-1034 (1985) re latent TGF- $\beta$  from chicken embryo fibroblasts; Oreffo et al., Biochem. Biophys. Res. Commun., **158**: 817-823 (1989) re latent TGF- $\beta$  from murine bone organ cultures; Keski-Oja et al., J. Cell Biol., **107**: (6 Part 3), 1988, 50a re latent TGF- $\beta$  from human lung adenocarcinoma cell line; Miyazono and Heldin, J. Cell. Biochem. Supp. **Q** (13 part B) 1989, p. 92 and Miyazono and Heldin, Nature, **338**: 158-160 (1989) re latent TGF- $\beta$  from human platelets and its carbohydrate structure; and Pircher et al., Biochem. Biophys. Res. Commun., **136**: 30-37 (1986) re latent TGF- $\beta$  from human blood platelets. See also Lawrence et al., J. Cell. Physiol., **121**: 184-188 (1984); Kryceve-Martinerie et al., Int. J. Cancer, **35**: 553-558 (1985); Brown et al., "TGF- $\beta$ ", NY Acad. Sci. Meeting Abstract, May 18-20, 1989; Danielpour et al., J. Cell. Physiol., **138**: 79-86 (1989); Wakefield et al., J. Biol. Chem., **263**: 7646-7654 (1988); and Miyazono et al., J. Biol. Chem., **263**: 6407-6415 (1988).

Several groups have characterized the latent form of TGF- $\beta$ 1 secreted by human platelets. Pircher et al., *supra*, stated that it has an apparent molecular weight of 400 Kd. More recently, it has been characterized as a three-component complex of about 235 Kd, wherein the active TGF- $\beta$ 1 (25 Kd dimer) is non-covalently associated with the remainder of the processed precursor (75 Kd dimer), which in turn is disulfide-bonded to an unrelated protein of 125-160 Kd (Wakefield et al., J. Biol. Chem., 263, *supra*; Miyazono et al., *supra*; Miyazono et al., J. Cell Biochem. Suppl., 0 (12 Part A), 1988, p. 200; Wakefield et al., J. Cell. Biochem. Suppl., 11A: 0, 46 (1987)).

The function of the binding protein of 125-160 Kd remains to be elucidated. Recent characterizations indicate that it contains at least 14 EGF-like repeats and six potential N-glycosylation sites and calcium binding domains (Kanzaki et al., "TGF- $\beta$ ", NY Acad. Sci. meeting abstract, May 18-20, 1989; Miyazono, "TGF- $\beta$ ", NY Acad. Sci. meeting abstract, May 18-20, 1989). Latent TGF- $\beta$  secreted by many cells in culture has a similar structure (Wakefield et al., J. Biol. Chem., *supra*), and this is the form in which TGF- $\beta$ 1 is probably perceived initially by target cells *in vivo*. It has been suggested that the precursor remainder of TGF- $\beta$  may have an important independent biological function based on conservation of sequences in the precursor region (Roberts et al., Recent Progress in Hormone Research, *supra*). Additionally, a mutation at position 33 of precursor TGF- $\beta$ 1 is reported to increase the yield of mature TGF- $\beta$ 1, and dimerization of the precursor "pro" region is suggested as necessary to confer latency (Brunner et al., J. Biol. Chem., 264: 13660-13664 (1989)).

Normal repair of tissue is a complex, sequential process involving many cell types. Fibroblasts, inflammatory cells, and keratinocytes all function in an integrated manner to promote cell division, differentiation, and migration. These processes in turn lead to enhanced connective tissue deposition and angiogenesis. Recent data suggest that these processes may be mediated both in an autocrine and paracrine manner by peptide growth factors such as TGF- $\beta$  (Postlethwaite et al., J. Exp. Med., 165: 251-256 (1987); Assoian et al., Nature, 308: 804-806 (1984)). Levels of endogenous TGF- $\beta$  have been reported to increase transiently in wound chambers of the rat (Cromack et al., J. Surg. Res., 42: 622-628 (1987)). Also, a crude extract of platelets containing multiple growth factors promoted healing of chronic skin ulcers (Knighton et al., Ann. Surg., 204: 322-330 (1986)). The results of these studies indirectly support the hypothesis that normal healing is mediated by locally produced peptide growth factors.

*In vivo*, TGF- $\beta$ 1 causes granulation tissue to form when injected intradermally (Roberts et al., Proc. Nat. Acad. Sci. USA, 83: 4167-4171 (1986); Sporn et al., Science, 219: 1329-1331 (1983)). *In vitro*, TGF- $\beta$ 1 stimulates the expression of fibronectin and collagen type I, in part mediated via increased levels of mRNA, and increases the deposition of fibronectin into the pericellular matrix (Wrana et al., Eur. J. Biochem., 159: 69-76 (1986); Ignatz and Massague, J. Biol. Chem., 261: 4337-4345 (1986); Fine and Goldstein, J. Biol. Chem., 262:

3897-3902 (1987); Ignatz et al., J. Biol. Chem., 262: 6443-6446 (1987); Raghoebar et al., J. Clin. Invest., 79: 1285-1288 (1987); Varga and Jimenez, Biochem. Biophys. Res. Commun., 138: 974-980 (1986)).

5 A single application of TGF- $\beta$  in collagen vehicle to incisions in normal rats significantly increased tensile strength compared with untreated or collagen vehicle treated incisions (Mustoe et al., Science, 237: 1333-1336 (1987)). See also Brown et al., Ann. Surg., 208: 788-794 (1988). In another study it was reported that TGF- $\beta$  treatment reversed doxorubicin depressed uptake of hydroxyproline and thymidine in wound chambers in rats, suggesting that TGF- $\beta$  might enhance the strength of the incisions by stimulating proliferation of cells and enhancing collagen synthesis (Grotendorst et al., J. Clin. Invest., 76: 2323-2329 (1985)).

10 These results were extended using an animal model that more closely approximates healing of surgical incisions (Curtsinger et al., Surgery, Gynecology & Obstetrics, 168: 517-522 (1989)). It was hypothesized that because TGF- $\beta$  is a potent chemoattractant for human fibroblasts (Postlethwaite et al., *supra*,) and stimulates collagen synthesis in cultures of renal fibroblasts in rats (Roberts et al., Proc. Natl. Acad. Sci. USA, *supra*), it may increase tensile strength by directly stimulating production of collagen by fibroblasts or by attracting inflammatory cells that may release peptide growth factors into the wounded area (Madtes et al., Cell, 53: 285-293 (1988); Morhenn, Immunol. Today, 9: 104-107 (1988)).

15 In addition to the scientific literature, the patent literature has also disclosed that TGF- $\beta$  is useful in treating existing traumata when administered systemically or applied topically to the traumatized tissue, with promotion of rapid proliferation of cells, particularly fibroblast cells (see, e.g., EP 128,849; EP 105,014; U.S. Pat. Nos. 4,843,063; 4,774,322; 4,774,228; and 4,810,691). There is, however, also a need for an agent that predisposes mammals to accelerated tissue repair before the mammals have been subjected to trauma.

20 Accordingly, it is an object of the present invention to provide a method for treating mammals that have not yet experienced tissue damage to promote accelerated proliferation of the cells surrounding the traumata and consequently rapid healing.

This object and other objects will become apparent to one of ordinary skill in the art.

#### Disclosure of Invention

30 This invention provides a method of predisposing a mammal to accelerated tissue repair comprising systemically administering to the mammal, prior to its exposure to tissue damage, an effective amount of TGF- $\beta$ . Preferably, the TGF- $\beta$  is administered no more than about 24 hours prior to the tissue damage exposure. More preferably, the TGF- $\beta$  is administered within a time range of from about 24 hours to greater than about 5 minutes before exposure to tissue damage.

35 Surprisingly, it has been found that administration of a single dose of TGF- $\beta$  systemically to a mammal at least 24 hours in advance of wounding accelerates healing of

the wound dramatically. This discovery was particularly surprising because TGF- $\beta$  has a circulating half-life in plasma of only about 5 minutes.

#### Brief Description of the Drawings

Figure 1 depicts the sequences of human TGF- $\beta$ 1, human TGF- $\beta$ 2, human TGF- $\beta$ 3, chick TGF- $\beta$ 4, and frog TGF- $\beta$ 5.

Figure 2 represents the peak load, which is a measure of strength, of linear skin incision wounds. Rats were treated intramuscularly with 5 mg prednisolone (asterisk) at the time of wounding to impair healing processes or treated with saline (black) as an unimpaired-healing control. Saline (diagonal stripes) or 10  $\mu$ g/kg rhTGF- $\beta$ 1 (cross-hatching) was administered intravenously 24 hours before (-24 hr.) or at the time of (0 hr.) wounding.

Figure 3 represents the peak load of the impaired-healing rat skin linear incision wounds treated intravenously with saline (black) or with 10  $\mu$ g/kg, 100  $\mu$ g/kg, or 500  $\mu$ g/kg of rhTGF- $\beta$ 1 and intramuscularly with 5 mg methylprednisolone at the time of wounding. A group treated with saline but not treated with methylprednisolone served as an unimpaired-healing control.

#### Description of the Preferred Embodiments

##### A. Definitions

As used herein, the term "TGF- $\beta$ " refers to the family of molecules described hereinabove, having the full-length, native amino acid sequence of any of the TGF- $\beta$ s from any species. Reference to such TGF- $\beta$  herein will be understood to be a reference to any one of the currently identified forms, including TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, TGF- $\beta$ 4, and TGF- $\beta$ 5 (whose sequences are shown in Figure 1), as well as to TGF- $\beta$  species identified in the future, including polypeptides derived from the sequence of any known TGF- $\beta$  and identical at 75% or more of the residues, their alleles, and their predetermined amino acid sequence variants, so long as they are effective in the method described herein. The specific terms "TGF- $\beta$ 1," "TGF- $\beta$ 2," and "TGF- $\beta$ 3" refer to the TGF- $\beta$ s defined in the literature, e.g., Derynck et al., *Nature*, *supra*, Seyedin et al., *J. Biol. Chem.*, *262*, *supra*, and deMartin et al., *supra*. In addition, the TGF- $\beta$  is suitably useful in the latent form or as an associated or unassociated complex of precursor and mature TGF- $\beta$ .

Members of the TGF- $\beta$  family are defined as those which have nine cysteine residues in the mature portion of the molecule, share at least 65% sequence identity with other known TGF- $\beta$  sequences in the mature region, and may compete for the same receptor. In addition, they all appear to be encoded as a larger precursor that shares a region of high homology near the N-terminus and shows conservation of three cysteine residues in the portion of the precursor that will later be removed by processing. Moreover, the TGF- $\beta$ s appear to have a processing site with four or five basic amino acids.

The TGF- $\beta$  is appropriately from any source, preferably mammalian, and most preferably human. TGF- $\beta$  from animals other than humans, for example, porcine or bovine

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sources, can be used for treating humans. Likewise, if it is desirable to treat other mammalian species such as domestic, farm, zoo, sports, or pet animals, human TGF- $\beta$ , as well as TGF- $\beta$  from other species, is suitably employed.

As used herein, the term "tissue damage" refers to any form of damage or trauma to soft or hard tissue, including thermally and/or mechanically induced trauma as well as damage caused by inflammatory, infectious, and immune responses. Examples of tissue damage include surgical incisions, such as internal and epidermal surgical incisions, and corneal surgery; burns, whether first, second, or third degree; bone damage such as bone fractures, bony defects, and prosthetic implants, including injury attendant surgery such as hip replacements; wounds, including lacerations, incisions, and penetrations; sites of expected development of ulcers such as, e.g., diabetic, dental, haemophiliac, varicose, or decubitus ulcers; chronic conditions or ulcers converted to acute wounds, preferably by surgery; infections of the bone such as osteomyelitis; and any inflammatory or immune response of soft tissue such as that seen with rheumatoid arthritis or any inflammatory condition leading to bone loss, whether infectious or non-infectious.

#### B. Modes for Carrying Out the Invention

The method of this invention involves systemic administration to a mammal, including domestic, farm, zoo, sports, or pet animals, but preferably a human, of an effective amount of TGF- $\beta$  as an agent that predisposes the tissue to accelerated repair.

The types of patients that may be treated by the method of this invention include not only those who do or would be expected to undergo normal tissue repair, but also those that would be predicted to or do exhibit abnormal tissue repair. Impaired wound healing has many causes, including diabetes, uremia, malnutrition, vitamin deficiencies, and systemic treatment with corticosteroids, radiation, or antineoplastic agents such as doxorubicin. Thus, this invention contemplates treatment of the latter as well as the former types of patients.

The TGF- $\beta$  molecule will be formulated and dosed in a fashion consistent with good medical practice taking into account the specific tissue involved, the condition of the individual patient, the site of delivery of the TGF- $\beta$ , the method of administration, and other factors known to practitioners. Thus, for purposes herein, the "therapeutically effective amount" of the TGF- $\beta$  is an amount that is effective to accelerate tissue repair in a mammal that undergoes tissue damage after administration of the TGF- $\beta$ .

The TGF- $\beta$  is prepared for storage or administration by mixing TGF- $\beta$  having the desired degree of purity with physiologically acceptable carriers, excipients, or stabilizers. Such materials are non-toxic to recipients at the dosages and concentrations employed. If the TGF- $\beta$  is water soluble, it may be formulated in a buffer such as acetate or other organic acid salt, preferably at a pH of about 5 to 6. If a TGF- $\beta$  variant is only partially soluble in water, it may be prepared as a microemulsion by formulating it with a nonionic surfactant such as Tween, Pluronic, or polyethylene glycol (PEG), e.g., Tween 80, in an amount of 0.04-0.05% (w/v),



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to increase its solubility. Optionally other ingredients may be added such as antioxidants, e.g., ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; chelating agents such as EDTA; and sugar alcohols such as mannitol or sorbitol.

The TGF- $\beta$  to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). The TGF- $\beta$  ordinarily will be stored in lyophilized form or as an aqueous solution since it is highly stable to thermal and oxidative denaturation. The pH of the TGF- $\beta$  preparations typically will be about 5, although higher or lower pH values may also be appropriate in certain instances. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of salts of the TGF- $\beta$ .

Therapeutic compositions containing the TGF- $\beta$  generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Sustained release formulations may also be prepared, and include the formation of microcapsular particles and implantable articles. For preparing sustained-release TGF- $\beta$  compositions, the TGF- $\beta$  is preferably incorporated into a biodegradable matrix or microcapsule. A suitable material for this purpose is a polylactide, although other polymers of poly-( $\alpha$ -hydroxycarboxylic acids), such as poly-D-(-)-3-hydroxybutyric acid (EP 133,988A), can be used. Other biodegradable polymers include poly(lactones), poly(acetals), poly(orthoesters), or poly(orthocarbonates). The initial consideration here must be that the carrier itself, or its degradation products, is nontoxic in the target tissue and will not further aggravate the condition. This can be determined by routine screening in representative animal models such as impaired rat skin linear incision models, or, if such models are unavailable, in normal animals.

For examples of sustained release compositions, see U.S. Patent No. 3,773,919, EP 58,481A, U.S. Patent No. 3,887,699, EP 158,277A, Canadian Patent No. 1176565, U. Sidman et al., Biopolymers, 22: 547 (1983), and R. Langer et al., Chem. Tech., 12: 98 (1982).

Tissue damage caused by infections may be treated with TGF- $\beta$  formulated with an effective amount of an antibiotic such as cephalosporin or penicillin. Alternatively, the antibiotic and TGF- $\beta$  may be administered separately to the patient using the general methods described above. The treating physician will be able to determine the proper dosages and administration routes of antibiotic based on conventional therapy for treating infectious conditions.

The dosage of TGF- $\beta$  to be employed is dependent upon the factors described above, especially the type of tissue damage which is expected. As a general proposition, a dose of

about 0.015 to 5 mg/kg, preferably to 0.5 mg/kg, of TGF- $\beta$  may be administered to the patient, whether via, e.g., one or more single administrations, continuous infusion, or bolus injection. The advantage of this invention lies in the use of only one administration of TGF- $\beta$ , preferably intravenously, so one dose is preferred. However, other dosage regimens may be useful. This administration takes place prior to infliction of damage to the tissue, e.g., before surgery, preferably no more than about 24 hours before tissue damage is inflicted, and more preferably from within 24 hours to greater than about 5 minutes prior to tissue damage.

The invention is more fully illustrated in the example set forth below, which is intended to represent one embodiment of the invention, but not the only embodiment.

#### EXAMPLE I

**Material:** Recombinant human TGF- $\beta$ 1 was cloned (Derynck et al., *Nature, supra*) and expressed in Chinese hamster ovary cells (using a method such as that described by Graycar et al., *Molecular Endocrinology*, 3: 1977-1986 (1989) and U.S. Pat. No. 4,886,747 issued December 12, 1989). The protein was purified by harvesting the cell culture fluid, concentrating this fluid with a Pellicon cassette system, diluting the concentrate with three vols. of a mixture of 50:1 of reagent alcohol to HCl, allowing the mixture to sit for 1 hour at 4°C, adjusting the pH to 7.5-8, centrifuging the mixture, loading the supernatant on a cation exchange S Sepharose Fast Flow column (previously equilibrated with 6 M urea, 20 mM MOPS buffer, pH 8), washing the column with the same buffer, eluting with a gradient of 0 to 0.4 M sodium chloride in the same buffer, making a pool from the gradient fractions run on a gel, adjusting the pH of the pool to 4.5, applying the pool to a second cation exchange SP Toyopearl column previously equilibrated in 2 M urea, 50 mM sodium acetate buffer at pH 4.5, washing the column with the same buffer, eluting with a gradient of 0 to 1 M sodium chloride in the same buffer, making a pool from the gradient fractions run on a gel, concentrating the pool on a stirred cell Amicon concentrator, loading the concentrate on a HW55S Toyopearl gel filtration column, washing with 1 M acetic acid, making a pool from the gradient fractions run on a gel, and exchanging the pool into 20 mM sodium acetate buffer at pH 5 over Cellufine GH-25.

Vehicle (saline) was formulated in the sodium acetate buffer at pH 5 without the TGF- $\beta$ 1. The material was stored at 4°C until use.

**Animal Surgery:** Adult male Sprague Dawley rats, 300-350 grams (Charles River Laboratories, Wilmington, MA), maintained in accordance with guidelines from the NIH and the American Association for the Accreditation of Laboratory Animal Care, were anesthetized by an intramuscular injection of ketamine hydrochloride/xylazine hydrochloride/acetylpromazine maleate mixture. The hair was clipped from the back and sides and disinfected with betadine and 70% alcohol rinse. At this time each rat was given a single intravenous (iv) injection of either saline or one of four concentrations of TGF- $\beta$ 1 at a volume of 1.0 ml/kg. After injection of vehicle or TGF- $\beta$ 1, two pairs of symmetrical

transverse full-thickness skin incisions approximately 2.5 cm in length were made by cutting through the subdermal panniculus carnosus musculature. Each wound was closed with two interrupted 4-0 stainless steel sutures evenly divided across the wound. After surgery each rat was administered either a single intramuscular injection of 5 mg methylprednisolone to inhibit inflammation and thus impair the healing process or saline to serve as an unimpaired healing control. The animals were returned to their cages and allowed to recover.

Additional animals were treated in an identical manner with the exception of a single intravenous dose of TGF- $\beta$ 1 administered 24, 48, or 72 hours before surgery rather than at the time of surgery.

**Tissue Sampling:** In a time-dependent manner rats were euthanized and 1-2 mm cross-sections of the wound from the center of each scar were removed with samples fixed in 10% neutral buffered formalin for light microscopic examination and Karnovsky's solution for electron microscopy. Two 8 x 25 mm samples from each wound were removed and fixed in 10% formalin for seven days for wound strength determinations.

**Tensometry:** Tissues were uniformly trimmed in width and length (8 mm x 25 mm) to assure that the edges of the scar were exposed on both sides of the sample. Tensometry was performed on coded samples using a calibrated tensometer (Instron Universal Testing Instrument Model 1011, Instron Corp., Canton, MA). The value determined was breaking strength (g), which is a measure of force in grams applied to the tissue at the point where the scar tissue visually breaks and a major deflection occurs in the tracing.

**Results:** Two separate studies were performed in which there were an unimpaired-healing control (saline) group and an impaired-healing control (saline) group and TGF- $\beta$ 1-treated group(s). The first study compared the effects of 10  $\mu$ g/kg TGF- $\beta$ 1 to saline control when administered intravenously either 24 hours prior to or just before skin incision. Results of this study are presented in Figure 2 and indicate that wounds treated with 10  $\mu$ g/kg TGF- $\beta$ 1 exhibited increased strength ( $p < 0.05$ ) compared to its concurrent vehicle control. In addition, the impaired-healing wounds treated with TGF- $\beta$ 1 were approximately 90% as strong as unimpaired-healing wounds treated with vehicle.

The second study was identical in design with the addition of 100  $\mu$ g/kg and 500  $\mu$ g/kg doses of TGF- $\beta$ 1. Results, presented in Figure 3, indicate that all three dose levels of TGF- $\beta$ 1 increased the strength of linear incision wounds compared with impaired-healing vehicle control ( $p < 0.01$ ). Both the 100 and 500  $\mu$ g/kg doses of TGF- $\beta$ 1 returned impaired-healing wounds to the same strength as unimpaired-healing vehicle treated wounds (Fig. 3).

Thus, TGF- $\beta$  is effective when administered as single iv doses of 10 to 500  $\mu$ g/kg in accelerating wound healing in this model. This model is predictive of the results that one would obtain in a clinical trial.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- 5 (i) APPLICANT: Genentech, Inc.
- (ii) TITLE OF INVENTION: Method of Predisposing Mammals to Accelerated Tissue Repair
- 10 (iii) NUMBER OF SEQUENCES: 5
- (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: Genentech, Inc.  
(B) STREET: 460 Point San Bruno Blvd  
(C) CITY: South San Francisco  
15 (D) STATE: California  
(E) COUNTRY: USA  
(F) ZIP: 94080
- 20 (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: patin (Genentech)
- 25 (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:
- 30 (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: U.S. Ser. No. 07/504,495  
(B) FILING DATE: 4 April 1990
- 35 (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Hasak, Janet E.  
(B) REGISTRATION NUMBER: 28,616  
(C) REFERENCE/DOCKET NUMBER: 637
- 40 (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: 415/266-1896  
(B) TELEFAX: 415/952-9881  
(C) TELEX: 910/371-7168

## (2) INFORMATION FOR SEQ ID NO:1:

- 45 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 390 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear
- 50 (iv) SEQUENCE DESCRIPTION:SEQ ID NO:1:
- Met Pro Pro Ser Gly Leu Arg Leu Leu Pro Leu Leu Leu Pro Leu  
1 5 10 15
- 55 Leu Trp Leu Leu Val Leu Thr Pro Gly Pro Pro Ala Ala Gly Leu

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	20	25	30
	Ser Thr Cys Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg		
	35	40	45
5	Ile Glu Ala Ile Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Ala		
	50	55	60
	Ser Pro Pro Ser Gln Gly Glu Val Pro Pro Gly Pro Leu Pro Glu		
10	65	70	75
	Ala Val Leu Ala Leu Tyr Asn Ser Thr Arg Asp Arg Val Ala Gly		
	80	85	90
15	Glu Ser Ala Glu Pro Glu Pro Glu Pro Glu Ala Asp Tyr Tyr Ala		
	95	100	105
	Lys Glu Val Thr Arg Val Leu Met Val Glu Thr His Asn Glu Ile		
	110	115	120
20	Tyr Asp Lys Phe Lys Gln Ser Thr His Ser Ile Tyr Met Phe Phe		
	125	130	135
	Asn Thr Ser Glu Leu Arg Glu Ala Val Pro Glu Pro Val Leu Leu		
25	140	145	150
	Ser Arg Ala Glu Leu Arg Leu Leu Arg Leu Lys Leu Lys Val Glu		
	155	160	165
30	Gln His Val Glu Leu Tyr Gln Lys Tyr Ser Asn Asn Ser Trp Arg		
	170	175	180
	Tyr Leu Ser Asn Arg Leu Leu Ala Pro Ser Asp Ser Pro Glu Trp		
	185	190	195
35	Leu Ser Phe Asp Val Thr Gly Val Val Arg Gln Trp Leu Ser Arg		
	200	205	210
	Gly Gly Glu Ile Glu Gly Phe Arg Leu Ser Ala His Cys Ser Cys		
40	215	220	225
	Asp Ser Arg Asp Asn Thr Leu Gln Val Asp Ile Asn Gly Phe Thr		
	230	235	240
45	Thr Gly Arg Arg Gly Asp Leu Ala Thr Ile His Gly Met Asn Arg		
	245	250	255
	Pro Phe Leu Leu Leu Met Ala Thr Pro Leu Glu Arg Ala Gln His		
	260	265	270
50	Leu Gln Ser Ser Arg His Arg Arg Ala Leu Asp Thr Asn Tyr Cys		
	275	280	285
	Phe Ser Ser Thr Glu Lys Asn Cys Cys Val Arg Gln Leu Tyr Ile		
55	290	295	300

	Asp	Phe	Arg	Lys	Asp	Leu	Gly	Trp	Lys	Trp	Ile	His	Glu	Pro	Lys
					305					310					315
5	Gly	Tyr	His	Ala	Asn	Phe	Cys	Leu	Gly	Pro	Cys	Pro	Tyr	Ile	Trp
					320					325					330
	Ser	Leu	Asp	Thr	Gln	Tyr	Ser	Lys	Val	Leu	Ala	Leu	Tyr	Asn	Gln
					335					340					345
10	His	Asn	Pro	Gly	Ala	Ser	Ala	Ala	Pro	Cys	Cys	Val	Pro	Gln	Ala
					350					355					360
	Leu	Glu	Pro	Leu	Pro	Ile	Val	Tyr	Tyr	Val	Gly	Arg	Lys	Pro	Lys
					365					370					375
15	Val	Glu	Gln	Leu	Ser	Asn	Met	Ile	Val	Arg	Ser	Cys	Lys	Cys	Ser
					380					385					390

25 (ii) **SEQUENCE CHARACTERISTICS:**  
 (A) **LENGTH:** 414 amino acids  
 (B) **TYPE:** amino acid  
 (D) **TOPOLOGY:** linear

30	Met	His	Tyr	Cys	Val	Leu	Ser	Ala	Phe	Leu	Ile	Leu	His	Leu	Val	
	1				5					10					15	
	Thr	Val	Ala	Leu	Ser	Leu	Ser	Thr	Cys	Ser	Thr	Leu	Asp	Met	Asp	
					20					25					30	
35	Gln	Phe	Met	Arg	Lys	Arg	Ile	Glu	Ala	Ile	Arg	Gly	Gln	Ile	Leu	
					35					40					45	
40	Ser	Lys	Leu	Lys	Leu	Thr	Ser	Pro	Pro	Glu	Asp	Tyr	Pro	Glu	Pro	
					50					55					60	
	Glu	Glu	Val	Pro	Pro	Glu	Val	Ile	Ser	Ile	Tyr	Asn	Ser	Thr	Arg	
					65					70					75	
45	Asp	Leu	Leu	Gln	Glu	Lys	Ala	Ser	Arg	Arg	Ala	Ala	Ala	Cys	Glu	
					80					85					90	
	Arg	Glu	Arg	Ser	Asp	Glu	Glu	Tyr	Tyr	Ala	Lys	Glu	Val	Tyr	Lys	
					95					100					105	
50	Ile	Asp	Met	Pro	Pro	Phe	Phe	Pro	Ser	Glu	His	Ala	Ile	Pro	Pro	
					110					115					120	
	Thr	Phe	Tyr	Arg	Pro	Tyr	Phe	Arg	Ile	Val	Arg	Phe	Asp	Val	Ser	
55					125					130					135	

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	Ala Met Glu Lys Asn Ala Ser Asn Leu Val Lys Ala Glu Phe Arg	140	145	150
5	Val Phe Arg Leu Gln Asn Pro Lys Ala Arg Val Pro Glu Gln Arg	155	160	165
	Ile Glu Leu Tyr Gln Ile Leu Lys Ser Lys Asp Leu Thr Ser Pro	170	175	180
10	Thr Gln Arg Tyr Ile Asp Ser Lys Val Val Lys Thr Arg Ala Glu	185	190	195
	Gly Glu Trp Leu Ser Phe Asp Val Thr Asp Ala Val His Glu Trp	200	205	210
15	Leu His His Lys Asp Arg Asn Leu Gly Phe Lys Ile Ser Leu His	215	220	225
	Cys Pro Cys Cys Thr Phe Val Pro Ser Asn Asn Tyr Ile Ile Pro	230	235	240
20	Asn Lys Ser Glu Glu Leu Glu Ala Arg Phe Ala Gly Ile Asp Gly	245	250	255
	Thr Ser Thr Tyr Thr Ser Gly Asp Gln Lys Thr Ile Lys Ser Thr	260	265	270
	Arg Lys Lys Asn Ser Gly Lys Thr Pro His Leu Leu Leu Met Leu	275	280	285
30	Leu Pro Ser Tyr Arg Leu Glu Ser Gln Gln Thr Asn Arg Arg Lys	290	295	300
	Lys Arg Ala Leu Asp Ala Ala Tyr Cys Phe Arg Asn Val Gln Asp	305	310	315
35	Asn Cys Cys Leu Arg Pro Leu Tyr Ile Asp Phe Lys Arg Asp Leu	320	325	330
	Gly Trp Lys Trp Ile His Glu Pro Lys Gly Tyr Asn Ala Asn Phe	335	340	345
	Cys Ala Gly Ala Cys Pro Tyr Leu Trp Ser Ser Asp Thr Gln His	350	355	360
45	Ser Arg Val Leu Ser Leu Tyr Asn Thr Ile Asn Pro Glu Ala Ser	365	370	375
	Ala Ser Pro Cys Cys Val Ser Gln Asp Leu Glu Pro Leu Thr Ile	380	385	390
50	Leu Tyr Tyr Ile Gly Lys Thr Pro Lys Ile Glu Gln Leu Ser Asn	395	400	405
55	Met Ile Val Lys Ser Cys Lys Cys Ser	410	414	

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## (2) INFORMATION FOR SEQ ID NO:3:

5 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 410 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

10 (iv) SEQUENCE DESCRIPTION:SEQ ID NO:3:

Met	His	Leu	Gln	Arg	Ala	Leu	Val	Val	Leu	Ala	Leu	Leu	Asn	Phe	1	5	10	15
Ala	Thr	Val	Ser	Leu	Ser	Leu	Ser	Thr	Cys	Thr	Thr	Leu	Asp	Phe	20	25	30	
Gly	His	Ile	Lys	Lys	Lys	Arg	Val	Glu	Ala	Ile	Arg	Gly	Gln	Ile	35	40	45	
Leu	Ser	Lys	Leu	Arg	Leu	Thr	Ser	Pro	Pro	Glu	Pro	Thr	Val	Met	50	55	60	
Thr	His	Val	Pro	Tyr	Gln	Val	Leu	Ala	Leu	Tyr	Asn	Ser	Thr	Arg	65	70	75	
Glu	Leu	Leu	Glu	Glu	His	Gly	Glu	Arg	Lys	Glu	Glu	Gly	Cys	Thr	80	85	90	
Gln	Glu	Asn	Thr	Glu	Ser	Glu	Tyr	Tyr	Ala	Lys	Glu	Ile	His	Lys	95	100	105	
Phe	Asp	Met	Ile	Gln	Gly	Leu	Ala	Glu	His	Asn	Glu	Leu	Ala	Val	110	115	120	
Cys	Pro	Lys	Gly	Ile	Thr	Ser	Lys	Val	Phe	Arg	Phe	Asn	Val	Ser	125	130	135	
Ser	Val	Glu	Lys	Asn	Arg	Thr	Asn	Leu	Phe	Arg	Ala	Glu	Phe	Arg	140	145	150	
Val	Leu	Arg	Val	Pro	Asn	Pro	Ser	Ser	Lys	Arg	Asn	Glu	Gln	Arg	155	160	165	
Ile	Glu	Leu	Phe	Gln	Ile	Leu	Arg	Pro	Asp	Glu	His	Ile	Ala	Lys	170	175	180	
Gln	Arg	Tyr	Ile	Gly	Gly	Lys	Asn	Leu	Pro	Thr	Arg	Gly	Thr	Ala	185	190	195	
Glu	Trp	Leu	Ser	Phe	Asp	Val	Thr	Asp	Thr	Val	Arg	Glu	Trp	Leu	200	205	210	
Leu	Arg	Arg	Glu	Ser	Asn	Leu	Gly	Leu	Glu	Ile	Ser	Ile	His	Cys	215	220	225	



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Pro Cys His Thr Phe Gln Pro Asn Gly Asp Ile Leu Glu Asn Ile  
 230 235 240  
 5 His Glu Val Met Glu Ile Lys Phe Lys Gly Val Asp Asn Glu Asp  
 245 250 255  
 Asp His Gly Arg Gly Asp Leu Gly Arg Leu Lys Lys Gln Lys Asp  
 260 265 270  
 10 His His Asn Pro His Leu Ile Leu Met Met Ile Pro Pro His Arg  
 275 280 285  
 Leu Asp Asn Pro Gly Gln Gly Gly Gln Arg Lys Lys Arg Ala Leu  
 290 295 300  
 15 Asp Thr Asn Tyr Cys Phe Arg Asn Leu Glu Glu Asn Cys Cys Val  
 305 310 315  
 Arg Pro Leu Tyr Ile Asp Phe Arg Gln Asp Leu Gly Trp Lys Trp  
 320 325 330  
 Val His Glu Pro Lys Gly Tyr Tyr Ala Asn Phe Cys Ser Gly Pro  
 335 340 345  
 25 Cys Pro Tyr Leu Arg Ser Ala Asp Thr Thr His Ser Thr Val Leu  
 350 355 360  
 Gly Leu Tyr Asn Thr Leu Asn Pro Glu Ala Ser Ala Ser Pro Cys  
 365 370 375  
 30 Cys Val Pro Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Val  
 380 385 390  
 Gly Arg Thr Pro Lys Val Glu Gln Leu Ser Asn Met Val Val Lys  
 395 400 405  
 35 Ser Cys Lys Cys Ser  
 410

40 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 304 amino acids

(B) TYPE: amino acid

45 (D) TOPOLOGY: linear

(iv) SEQUENCE DESCRIPTION:SEQ ID NO:4:

Met Asp Pro Met Ser Ile Gly Pro Lys Ser Cys Gly Gly Ser Pro  
 1 5 10 15  
 Trp Arg Pro Pro Gly Thr Ala Pro Trp Ser Ile Gly Ser Arg Arg  
 20 25 30

55

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	Ala Thr Ala Ser Ser Ser Cys Ser Thr Ser Ser Arg Val Arg Ala	35	40	45
5	Glu Val Gly Gly Arg Ala Leu Leu His Arg Ala Glu Leu Arg Met	50	55	60
	Leu Arg Gln Lys Ala Ala Ala Asp Ser Ala Gly Thr Glu Gln Arg	65	70	75
10	Leu Glu Leu Tyr Gln Gly Tyr Gly Asn Ala Ser Trp Arg Tyr Leu	80	85	90
	His Gly Arg Ser Val Arg Ala Thr Ala Asp Asp Glu Trp Leu Ser	95	100	105
15	Phe Asp Val Thr Asp Ala Val His Gln Trp Leu Ser Gly Ser Glu	110	115	120
	Leu Leu Gly Val Phe Lys Leu Ser Val His Cys Pro Cys Glu Met	125	130	135
20	Gly Pro Gly His Ala Asp Glu Met Arg Ile Ser Ile Glu Gly Phe	140	145	150
	Glu Gln Gln Arg Gly Asp Met Gln Ser Ile Ala Lys Lys His Arg	155	160	165
	Arg Val Pro Tyr Val Leu Ala Met Ala Leu Pro Ala Glu Arg Ala	170	175	180
30	Asn Glu Leu His Ser Ala Arg Arg Arg Arg Asp Leu Asp Thr Asp	185	190	195
	Tyr Cys Phe Gly Pro Gly Thr Asp Glu Lys Asn Cys Cys Val Arg	200	205	210
35	Pro Leu Tyr Ile Asp Phe Arg Lys Asp Leu Gln Trp Lys Trp Ile	215	220	225
	His Glu Pro Lys Gly Tyr Met Ala Asn Phe Cys Met Gly Pro Cys	230	235	240
	Pro Tyr Ile Trp Ser Ala Asp Thr Gln Tyr Thr Lys Val Leu Ala	245	250	255
45	Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala Ala Pro Cys Cys	260	265	270
	Val Pro Gln Thr Leu Asp Pro Leu Pro Ile Ile Tyr Tyr Val Gly	275	280	285
50	Arg Asn Val Arg Val Glu Gln Leu Ser Asn Met Val Val Arg Ala	290	295	300
55	Cys Lys Cys Ser	304		

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## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 198 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (iv) SEQUENCE DESCRIPTION:SEQ ID NO:5:

10	Asp	Glu	Trp	Met	Ser	Phe	Asp	Val	Thr	Lys	Thr	Val	Asn	Glu	Trp	1	5	10	15
	Leu	Lys	Arg	Ala	Glu	Glu	Asn	Glu	Gln	Phe	Gly	Leu	Gln	Pro	Ala	20	25	30	
15	Cys	Lys	Cys	Pro	Thr	Pro	Gln	Ala	Lys	Asp	Ile	Asp	Ile	Glu	Gly	35	40	45	
20	Phe	Pro	Ala	Leu	Arg	Gly	Asp	Leu	Ala	Ser	Leu	Ser	Ser	Lys	Glu	50	55	60	
	Asn	Thr	Lys	Pro	Tyr	Leu	Met	Ile	Thr	Ser	Met	Pro	Ala	Glu	Arg	65	70	75	
25	Ile	Asp	Thr	Val	Thr	Ser	Ser	Arg	Lys	Lys	Arg	Gly	Val	Gly	Gln	80	85	90	
	Glu	Tyr	Cys	Phe	Gly	Asn	Asn	Gly	Pro	Asn	Cys	Cys	Val	Lys	Pro	95	100	105	
30	Leu	Tyr	Ile	Asn	Phe	Arg	Lys	Asp	Leu	Gly	Trp	Lys	Trp	Ile	His	110	115	120	
	Glu	Pro	Lys	Gly	Tyr	Glu	Ala	Asn	Tyr	Cys	Leu	Gly	Asn	Cys	Pro	125	130	135	
35	Tyr	Ile	Trp	Ser	Met	Asp	Thr	Gln	Tyr	Ser	Lys	Val	Leu	Ser	Leu	140	145	150	
40	Tyr	Asn	Gln	Asn	Asn	Pro	Gly	Ala	Ser	Ile	Ser	Pro	Cys	Cys	Val	155	160	165	
	Pro	Asp	Val	Leu	Glu	Pro	Leu	Pro	Ile	Ile	Tyr	Tyr	Val	Gly	Arg	170	175	180	
45	Thr	Ala	Lys	Val	Glu	Gln	Leu	Ser	Asn	Met	Val	Val	Arg	Ser	Cys	185	190	195	
50	Asn	Cys	Ser													198			

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Claims

1. A method of predisposing a mammal to accelerated tissue repair comprising systemically administering to the mammal, prior to its exposure to tissue damage, an effective amount of TGF- $\beta$ .
- 5        2. The method of claim 1 wherein the TGF- $\beta$  is administered no more than about 24 hours prior to the tissue damage exposure.
3. The method of claim 1 wherein the TGF- $\beta$  is administered within a time range of from about 24 hours to greater than about 5 minutes prior to the tissue damage exposure.
4. The method of claim 1 wherein the administration is in a single dose.
- 10       5. The method of claim 1 wherein the administration is intravenous.
6. The method of claim 1 wherein the TGF- $\beta$  is human TGF- $\beta$ .
7. The method of claim 6 wherein the TGF- $\beta$  is TGF- $\beta$ 1.
8. The method of claim 1 wherein the mammal is human.
9. The method of claim 1 wherein the tissue repair is wound healing and the tissue  
15 damage is a wound.
10. The method of claim 1 wherein the tissue repair is bone repair and the tissue damage is a bone fracture, prosthetic implant, or bony defect.
11. The method of claim 1 wherein the tissue damage is surgical incision.

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	1	10	20	32	40	50
Hu TGF- $\beta$	1	MP	PSGLRL	LLPL	LLWLV	-LTPGPPAAGLSTCKTIDMELVKRKRIEAIR
Hu TGF- $\beta$	2	MH	YCVLSAFL	ILHLV	---TVAL	-----S-LSTCSTLDMQDFMRKRIEAIR
Hu TGF- $\beta$	3	---	MHLQ	RALV	VLALLNFATVSL	-----S-LSTCTTDLDFGHKKRVEAIR
		60	70	80	90	
Hu TGF- $\beta$	1	GQ	ILSKLRLASPPSQGE	-VP	-PGPLPEAVLALYNSTRDRVAGESAEPE	-PE
Hu TGF- $\beta$	2	GQ	ILSKLKLTSPP	---EDY	PEPEEVPPEVISIYNSTRDLL	---QEKASR-RA
Hu TGF- $\beta$	3	GQ	ILSKLRLTSPP	---EPTV	-MTHVPYQVLALYNSTRELL	---EEHGER-KE
Ck TGF- $\beta$	4	---	-----	-----	-----M	---DPMSIGPK-
		100	110	120	130	
Hu TGF- $\beta$	1	P	-----	EADY	YAKEVTRVLMV	---ETHNEIYDKFKQSTHSIYFFNTS
Hu TGF- $\beta$	2	A	A	CERERSDEEY	YAKEVYKIDMPFFPS	-EHAIPPTFYRPY-FRIVRFDVS
Hu TGF- $\beta$	3	E	GCTQ	ENTESEY	YAKEIHKFDMIQGLAE	-HNELAVCPKGIT-SKVFRFNVS
Ck TGF- $\beta$	4	-	SCG	-----	-----GSPW	-RPP-GTAPWSIG-SR---RATAS
		140	150	160	170	
Hu TGF- $\beta$	1	EL	-----	RE	-AVPEP	VLLS-RAELRLRLKL---KV-EQHVELYQ-----
Hu TGF- $\beta$	2	A	-----	MEK	NASNLV-KAEFR	VFRVLQNP
Hu TGF- $\beta$	3	S	-----	VEK	NRTNLF-RAEFR	VLRVPNPS-SKRNEQRIELFQILRP-
Ck TGF- $\beta$	4	SS	CTSS	RVRAEV	GGRRALLHRAEL	RMLRQKAAADSAGTEQRLELYQGYGN-
		180	190	200	210	220
Hu TGF- $\beta$	1	K	YSNN	SWRYLSNR	LLAPSD	SPWL
Hu TGF- $\beta$	2	DL	TSP	TQRYID	SKVKT	RAEGEW
Hu TGF- $\beta$	3	DE	HI	AQRYI	GGKNL	PTRGTA
Ck TGF- $\beta$	4	---	AS	WRYLHGR	SVRATA	DEWL
Fg TGF- $\beta$	5	---	---	---	---	---

FIG. 1A

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Hu TGF- $\beta$  1 SC-----DSRDNTLQVDIN-GFTTGR-----RGDLATI-----250  
Hu TGF- $\beta$  2 PCCTFVPSNNYIIPNKSEEEARFA-GIDGTSTVTSGDQKTIKSTRKNSG  
Hu TGF- $\beta$  3 PCHTFQP-NGDILENIHEVMEIKFK-GVDNEDDHGRDGLRLK---KQKDH  
Ck TGF- $\beta$  4 PCEMGPG-HADEMRISIEGFEQQ-----RGDMQSI---K-KHR  
Fg TGF- $\beta$  5 KCPT-PQ-AKDI-D--IEGFPAL-----RGDLASLSS--KEN--

260 270 280 290  
Hu TGF- $\beta$  1 HGMNRPFLLLMATPLERA-QH--LQSS----RHRRALDTNYCF--SSTEKNC  
Hu TGF- $\beta$  2 KT---PHLLMLLPSYRL-ESQ-----QTNRRKKRALDAAYCF--RNVQDNC  
Hu TGF- $\beta$  3 H--N-PHLLIMMIPPHRL-DNPGQGGQ---RKKRALDTNYCF--RNLEENC  
Ck TGF- $\beta$  4 R--V-PYVLAMALPAERANE---LHSA---RRRRDLTDYCFGPGTDEKNC  
Fg TGF- $\beta$  5 -TK--PYL--MIT-SMPAERIDVTSS---RKKRGVGQEYCF--GNNGPNC

300 310 320 330 340  
Hu TGF- $\beta$  1 CVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN  
Hu TGF- $\beta$  2 CLRPLYIDFKRDLGWKWIHEPKGYNANFCAGACPYLWSSDTQHSRVLALYN  
Hu TGF- $\beta$  3 CVRPLYIDFRQDLGWKWWHEPKGYVANFCSGPCPYLRSADTHSTVLGLYN  
Ck TGF- $\beta$  4 CVRPLYIDFRKDLQWKWIHEPKGYMANFCMGPCPYIWSADTQYTKVLALYN  
Fg TGF- $\beta$  5 CVKPLYINFRKDLGWKWIHEPKGYEANYCLGNCPYIWSMDTQYSKVLALYN

350 360 370 380 390  
Hu TGF- $\beta$  1 QHNPASAAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS  
Hu TGF- $\beta$  2 TINPEASASPCCVSQDLEPLTILYVIGKTPKIEQLSNMIVKSKCS  
Hu TGF- $\beta$  3 TLNPEASASPCCVQDLEPLTILYVYVGRTPKVEQLSNMVVKSKCS  
Ck TGF- $\beta$  4 QHNPASAAAPCCVPQTLDPPLPIIYVYVGRVNRVEQLSNMVVRACKCS  
Fg TGF- $\beta$  5 QNNPGASISPCCVDPVLEPLPIIYVYVGRVNRVEQLSNMVVRSCNCS

FIG. 1B

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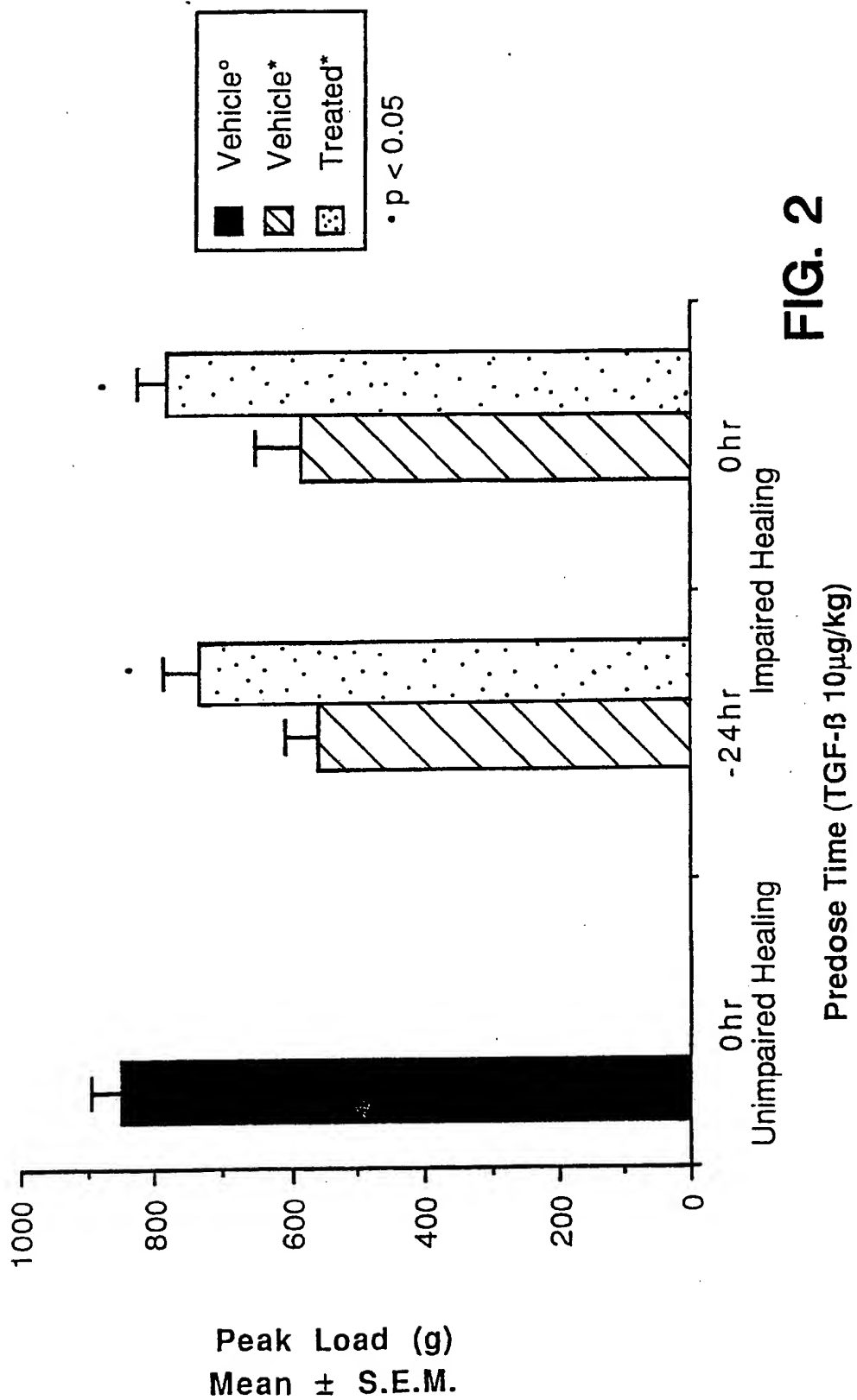


FIG. 2

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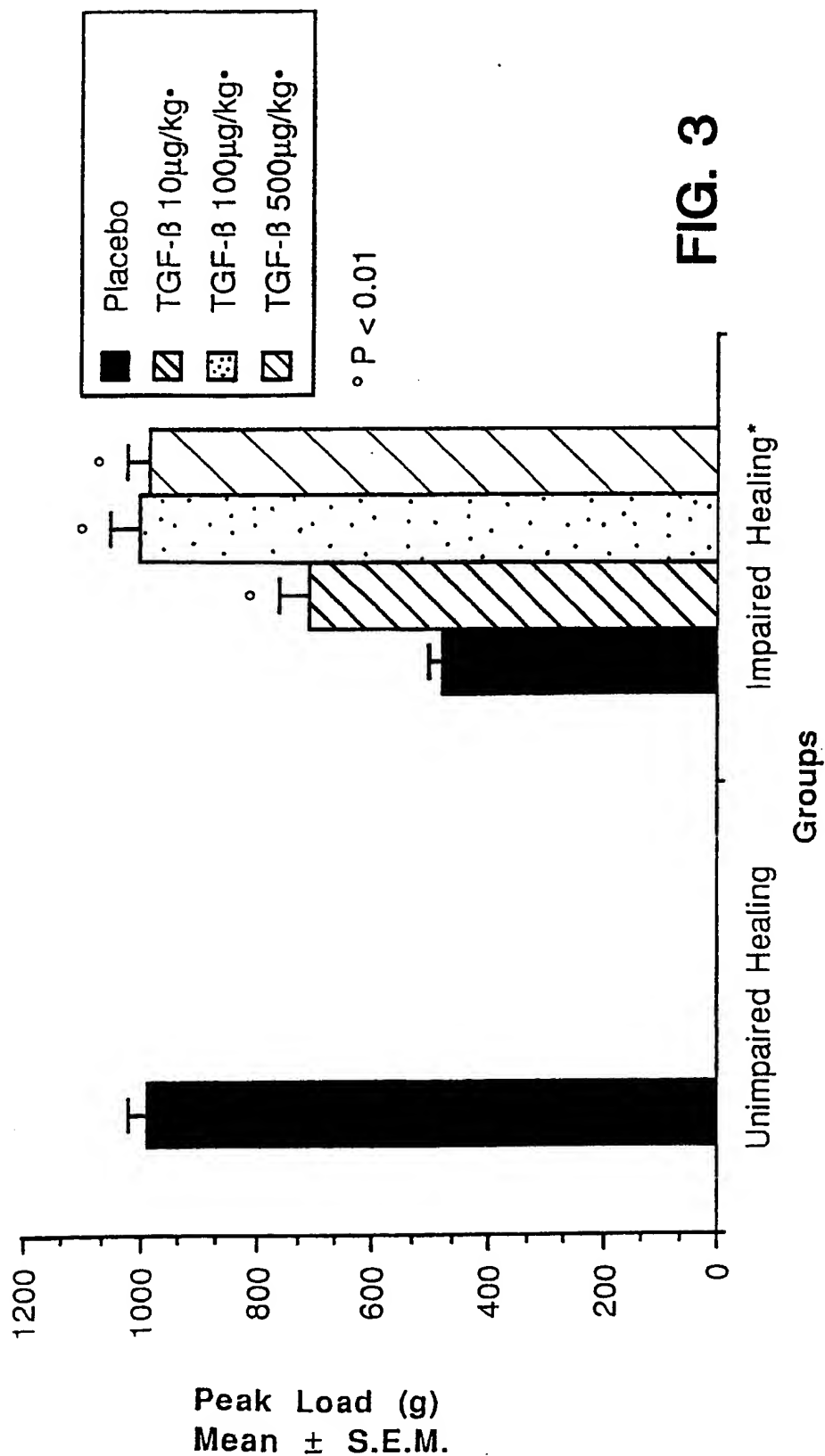


FIG. 3



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